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Effect of straw size and microbial amendment of litter on certain litter quality parameters, ammonia emission, and footpad dermatitis in broilers

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Abstract. This study was conducted to evaluate the impact of litter amendment (microbiological product – Micropan complex) and straw size (unchopped and chopped straw) on pH, moisture level, ammonia emission, and footpad dermatitis in broilers. A total of 1200 1-day-old Ross 308 broilers were randomly allocated to four treatments (2×2 factorial arrangement), with four replicates per treatment. Each replicate consisted of 75 as-hatched birds per pen. The first factor consisted of wheat straw – chopped (C) or unchopped (U) – and the second factor was the litter amendment with (WM) or without (OM) Micropan® (enzymatic bacterial product – Eurovix, USA). At 4, 5, and 6 weeks of the trial, the levels of ammonia concentration, moisture content, and pH of litter in each pen were measured. The foot pad lesions were evaluated at 21, 35, and 42 days of age. Broilers reared on chopped straw had significantly better leg condition as expressed by a smaller incidence and severity of footpad dermatitis. The pH value of litter was decreased and footpad quality was significantly improved by Micropan application in broilers kept on chopped and unchopped straw in the sixth week of age. There was no significant effect of Micropan application and straw size on ammonia emission in broiler housing.

1 Introduction

The quality of litter is a very significant factor in rearing broilers from the age of 1 day until slaughter because it affects health, productive parameters, carcass quality, and the welfare of broilers. According to Shepherd and Fairchild (2010), Grimes et al. (2002), and Bilgili et al. (2009), litter has several purposes, such as thermal insulation, moisture absorption, and acting as a protective barrier from the ground, and it allows bird scratching.

Several materials have been examined for use as broiler litter (rice hulls, ground corncobs, stump chips, pine sawdust, wood shavings, bark and chips, pine bark, clay sand, coconut husk, Guinea grass, newspaper, corn cob, wheat straw, ground rapeseed straw, and silage maize (Grimes et al., 2002; Sirri et al., 2007; Meluzzi et al., 2008; van Harn et al., 2012; Garcês et al., 2013). Each of these materials has its advantages and disadvantages, but the different particle size of these materials was seen to be the most important factor regarding paw quality and has been examined as a contributing factor in the development of footpad dermatitis (FPD) (Grimes et al., 2002).

While the most commonly used litter material in America is pine shavings, in Europe it is straw (Grimes et al., 2002; Shepherd and Fairchild, 2010). Serbia produces a lot of wheat and barley, whose straw is mostly used as a litter in broiler production. The availability and lower price of the straw compared to the wood shavings has led to a very intensive use of the straw as a bedding material, despite of its rather poor characteristics and negative influence on the development of FPD (Sirri et al., 2007; Meluzzi et al., 2008). Although there is some evidence that the particle size of the bedding has a significant effect on FPD (Grimes et al., 2002), further evidence is needed to confirm that the chopping of the straw could positively influence the condition of footpads.

Ammonia (NH_3) emissions from broiler litter have several disadvantages, and recommended concentrations are 25 to 50 ppm in broiler houses (Miles et al., 2004). On the one hand, it causes environmental problems, while on the other hand, it has a negative effect on the health, welfare, and per-

formance of birds (Miles et al., 2004; Atapattu et al., 2008; Stokstad, 2014). NH_3 formation is directly controlled by factors such as pH, temperature, and the moisture level in the litter (Elliott and Collins, 1982; Carr et al., 1990). Ammonia in the litter may contribute to further development of FPD (Haslam et al., 2006; Bilgili et al., 2009), although it does not seem to directly cause it (Martins et al., 2013). The bacteria-generated ammonia dissolves at a high moisture level (Mayne et al., 2007; Meluzzi et al., 2008; Allain et al., 2009; Nowaczewski et al., 2011) and forms an alkaline solution that acts as an irritant to the footpads.

Various litter amendments, such as chemicals – aluminium sulfate (Zhang et al., 2011), sodium bisulfate (Nagaraj et al., 2007; Li et al., 2012) – dietary manipulations (Ferguson et al., 1998; Eichner et al., 2007), zeolite (Li et al., 2008), microbiological preparation (Iwańczuk-Czernik et al., 2007), and a commercial ammonia binding agent (Lazarevic et al., 2014), are used to reduce litter moisture, pH, and NH_3 emission in boiler houses. Litter treatments with chemical or microbiological products have positive effects on litter condition by lowering the pH (Zhang et al., 2011), and reducing ammonia emission (Iwańczuk-Czernik et al., 2007; Li et al., 2008; Zhang et al., 2011) or litter moisture (Iwańczuk-Czernik et al., 2007; Lazarevic et al., 2014).

This study was conducted to evaluate the impact of the straw size and the litter amendment (microbiological product – Micropan complex) on litter quality parameters (pH and moisture levels), ammonia emission, and footpad dermatitis in broilers.

2 Materials and methods

2.1 Experimental design and husbandry

The experiment was conducted at the experimental farm of the Faculty of Agriculture in Novi Sad, Serbia. A total of 1200 1-day-old Ross 308 broilers were randomly allocated to four treatments (2×2 factorial arrangement), with four replicates per treatment. Each replicate consisted of 75 as-hatched birds housed from 1-day old in floor pens measuring 5 m^2 with stocking density of 15 birds m^{-2}. The basic environmental parameters were in accordance with the demands of the hybrid used (Aviagen, 2014) with a lighting program of 18 h light and 6 h dark. Birds were vaccinated against Newcastle disease (NCD) and infectious bursal disease (IBD) as required by the country's veterinary authority. Feed and water were available ad libitum.

Broilers were reared to 42 days of age on a three-phase commercial feeding program consisting of a starter diet between 0 and 3 weeks (22.00 % crude protein, 12.65 ME MJ kg^{-1}), a grower diet between 3 and 5 weeks (21.00 % crude protein, 13.20 ME MJ kg^{-1}), and a finisher diet in the sixth week (19.00 % crude protein, 13.40 ME MJ kg^{-1}).

The experimental treatments consisted of two factors. The first factor was related to the size of wheat straw – unchopped (U) or chopped (C) – and the second factor was the litter treatment with an enzymatic bacterial product (Micropan® Simplex, Eurovix USA), which was applied on both chopped (WM) and unchopped straw (OM). The straw chopping was performed mechanically, and the length of the cut straw was ca. 20 mm, with 2.5 kg of straw used per m^2 of floor area. The product Micropan contains microorganisms and enzyme components and is used for the deodorization and sanification of poultry litter and manure. Micropan was applied to the surface of the litter according to the manufacturer's recommended rate (1 kg 100 m^{-2}) at the beginning of the cycle, and further treatments took place on days 15 and 35 of the trial.

All procedures were conducted according to ethical norms proposed by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, confirmed by Serbian authorities (Službeni glasnik RS – Međunarodni ugovori, 1/2010).

2.2 Sample analysis

2.2.1 Litter measurements

The litter moisture content was determined by the loss of weight after drying. Moisture was measured from the samples taken from the five different sites per pen (four from each corner and one from the centre). All five samples were mixed before analysis.In the lab, the pooled litter was weighed and dried at 105 °C for 24 h and weighed again. The decrease in weight was recorded as moisture content (%).

For determining the pH, 10 g litter samples from five areas of each pen were taken and immersed in 100 mL of distilled water. A weighted sample mat was then stirred for 15 min in a magnetic stirrer; thereafter, the sample was filtered through filter paper. After completion of filtration, the pH in the filtered liquid was measured using a pH meter (Inolab 720, WTW, Germany).

At 4, 5, and 6 weeks of the trial, the levels of ammonia concentration, moisture content, and pH of litter in each pen were measured. Ammonia concentration was determined by the method of Bilgili et al. (2009) using Dräger tubes with a measuring range from 5 to 70 ppm (5/a) and a Dräger Accuro pump. The measurement was done in the following manner: a certain part of litter was covered with plastic containers ($40.5 \times 31.5 \times 12$ cm, with a volume of 0.15 m^2) for 60 s, and after that, the concentration of ammonia was measured and read by inserting a measuring tube in the opening of the plastic containers located at the top centre.

2.2.2 FPD scoring

The incidence and severity of FPD were measured at 21, 35, and 42 days of age by the method described by Martrenchar et al. (2002), examining the left and right feet of birds. Birds with very dirty paws had their paws cleaned with a paper tissue before scoring. The footpad lesions were assigned to

Table 1. Moisture content (%) and pH of litter (unchopped or chopped straw) with or without addition of Micropanin in the fourth, fifth, and sixth week of age ($\bar{x} \pm$ SD).

		Unchopped straw		Chopped straw		*P*		
	Weeks	UOM	UWM	COM	CWM	Litter	Micropan	Interaction
Moisture	Fourth week	27.03^{a} ±4.60	25.53^{a} ±1.86	46.28^{b} ±11.27	47.16^{b} ±12.57	0.001	0.945	0.791
	Fifth week	38.67 ±8.51	38.26 ±11.23	43.14 ±4.30	45.31 ±5.45	0.169	0.826	0.748
	Sixth week	48.43 ±12.25	48.96 ±6.32	47.44 ±5.98	51.76 ±3.56	0.819	0.542	0.633
pH	Fourth week	8.82 ±0.18	8.80 ±0.18	8.70 ±0.18	8.69 ±0.23	0.285	0.884	1.000
	Fifth week	8.66^{b} ±0.41	7.68^{a} ±0.32	8.70^{b} ±0.53	7.55^{a} ±0.14	0.824	0.000	0.711
	Sixth week	8.72 ±0.32	8.90 ±0.18	8.77 ±0.13	8.91 ±0.29	0.843	0.228	0.859

$^{a-b}$ Means within the same rows with different superscripts are significantly different ($P < 0.05$); UOM: unchopped straw without addition of Micropan; UWM: unchopped straw with addition of Micropan; COM: chopped straw without addition of Micropan; CWM: chopped straw with addition of Micropan.

Table 2. Effect of straw size and litter amendment (Micropan) on ammonia concentration (NH_3, ppm) in the fourth, fifth, and sixth week of age ($\bar{x} \pm$ SD).

Treatment	Unchopped straw		Chopped straw		*P*		
Weeks	UOM	UWM	COM	CWM	Litter	Micropan	Interaction
Fourth week	36.88 ±5.25	43.44 ±21.83	60.31 ±15.76	60.31 ±26.46	0.056	0.737	0.737
Fifth week	51.25 ±22.78	58.75 ±34.25	62.50 ±23.00	51.88 ±18.64	0.866	0.904	0.488
Sixth week	57.65 ±5.73	59.90 ±20.42	64.15 ±21.93	63.75 ±30.63	0.366	0.707	0.264

UOM: unchopped straw without addition of Micropan; UWM: unchopped straw with addition of Micropan; COM: chopped straw without addition of Micropan; CWM: chopped straw with addition of Micropan.

one of four scores: no lesions (score 0), lesions in some areas (< 25 %) (score 1), lesions in wide areas (between 25 and 50 %) (score 2), and more than 50 % lesion on the footpads (score 3). The mean score of FPD was calculated as the cumulative total of the lesion scores divided by the total number of birds examined.

2.2.3 Statistical analyses

Data were analysed by factorial analysis of variance (ANOVA) using the GLM (general linear model) procedure. Means were separated by a Duncan post hoc test using StatSoft computer package (STATISTICA 11, 2012). Results were considered significant when $P < 0.05$.

3 Results

The mean values of the litter moisture content and pH for different groups is presented in Table 1. The moisture content was significantly lower in groups with unchopped straw in the fourth week of age, but towards the end of the trial this effect was lost. In the fifth and sixth week of age, there were no significant differences between groups in moisture content in the litter. In the same period, the application of Micropan did not affect the moisture content. At the fifth and sixth week of age, it was noticed that a crust formed on the surface of the litter treated with Micropan in both chopped and unchopped straw litter pens.

Measurement of pH in the fifth week of age indicated that the application of Micropan significantly reduced pH in both types of the straw (chopped and unchopped). However, in the fourth and sixth week of age, neither the size of the straw nor litter amendment influenced the pH value in the litter.

Table 3. Effect of straw size and litter amendment (Micropan) on the incidence of foot pad dermatitis (FPD) in broilers at 3 weeks of age ($\bar{x} \pm$ SD).

Treatment	Unchopped straw				Chopped straw				*P*		
Parameter	UOM		UWM		COM		CWM				
Lesions	No.	%	No.	%	No.	%	No.	%	Litter	Micropan	Interaction
0	4	1.99	9	4.57	32	15.85	48	24.87			
1	151	74.75	107	54.31	143	70.79	136	70.46			
2	47	23.26	65	32.99	27	13.36	9	4.66			
3	0	0.00	16	8.13	0	0.00	0	0.00			
Total	202	100.00	197	100.00	202	100.00	193	100.00			
Average score	1.21^{c} ±0.45		1.44^{d} ±0.70		0.97^{b} ±0.54		0.79^{a} ±0.50		0.000	0.478	0.000

[a–d] Means within the same rows with different superscripts are significantly different ($P < 0.05$); COM: chopped straw without addition of Micropan; CWM: chopped straw with addition of Micropan; UOM: unchopped straw without addition of Micropan; UWM: unchopped straw with addition of Micropan.

Table 4. Effect of straw size and litter amendment (Micropan) on the incidence of foot pad dermatitis (FPD) of broilers at 5 weeks of age ($\bar{x} \pm$ SD).

Treatment	Unchopped straw				Chopped straw				*P*		
Parameter	UOM		UWM		COM		CWM				
Lesions	No.	%	No.	%	No.	%	No.	%	Litter	Micropan	Interaction
0	14	7.07	12	5.83	17	7.91	25	12.20			
1	49	24.75	66	32.04	90	41.86	75	36.59			
2	66	33.33	70	33.98	62	28.84	44	21.46			
3	69	34.85	58	28.16	46	21.40	61	29.76			
Total	198	100.00	206	100.00	215	100.00	205	100.00			
Average score	1.95^{c} ±0.93		1.84^{bc} ±0.90		1.63^{a} ±0.90		1.68^{ab} ±1.02		0.000	0.625	0.209

[a–c] Means within the same rows with different superscripts are significantly different ($P < 0.05$); UOM: unchopped straw without addition of Micropan; UWM: unchopped straw with addition of Micropan; COM: chopped straw without addition of Micropan; CWM: chopped straw with addition of Micropan.

There were no significant interactions between litter size and Micropan application at any age of the birds.

Table 2 shows the effect of straw size and Micropan on ammonia concentration (NH_3, ppm) in the fourth, fifth, and sixth week of age. In all 3 observed weeks ammonia concentration was not significantly influenced by the addition of Micropan or the straw size, and the interaction between litter size and Micropan was not significant.

Footpad lesions were evaluated at 21, 35, and 42 days of age. The incidence and severity of footpad lesions of broilers by weeks are presented in Tables 3, 4, and 5. The influence of the litter type on the average FPD score was highly significant ($P < 0.01$). Broiler chickens kept on chopped straw had significantly better paw condition as expressed by a smaller incidence and severity of FPD at all weeks of age. On the other hand, addition of Micropan showed a significant effect on FPD score ($P < 0.01$) only at 6 weeks of age. The interaction of litter and Micropan was significant only at 3 weeks of age. The addition of Micropan significantly reduced the incidence of FPD in broilers raised on chopped straw but not on the unchopped straw as revealed by the significant interaction.

4 Discussion

Several studies have shown that FPD can be induced by litter moisture alone (Mayne et al., 2007; Meluzzi et al., 2008; Allain et al., 2009; Nowaczewski et al., 2011). On the other hand, Eichner et al. (2007) and Nagaraj et al. (2007) have found no significant correlation between litter moisture and the incidence and severity of FPD, which coincides with our results. In the fifth week of age, the application of Micropan led to the lowering of the pH with both types litter (chopped and unchopped). Although the pH of the litter treated with

Table 5. Effect of straw size and litter amendment (Micropan) on the incidence of foot pad dermatitis (FPD) in broilers at 6 weeks of age ($\bar{x} \pm$ SD).

Treatment	Unchopped straw				Chopped straw				*P*		
Parameter	UOM		UWM		COM		CWM				
Lesions	No.	%	No.	%	No.	%	No.	%	Litter	Micropan	Interaction
0	5	2.69	3	1.48	3	1.50	26	14.44			
1	57	30.65	86	42.36	65	32.50	77	42.78			
2	57	30.65	70	34.48	66	33.00	47	26.11			
3	67	36.02	44	21.67	66	33.00	30	16.67			
Total	186	100.00	203	100.00	200	100.00	180	100.00			
Average score	$1.94^{c} \pm 0.92$		$1.76^{b} \pm 0.80$		$1.71^{b} \pm 0.84$		$1.47^{a} \pm 0.93$		0.000	0.000	0.530

$^{a-c}$ Means within the same rows with different superscripts are significantly different ($P < 0.05$); UOM: unchopped straw without addition of Micropan; UWM: unchopped straw with addition of Micropan; COM: chopped straw without addition of Micropan; CWM: chopped straw with addition of Micropan.

Micropan was significantly lower in week 5 compared to untreated groups, this did not lead to the lowering of concentration of ammonia in the litter. That could be explained by the fact that the moisture content in the litter was very high in all groups, and NH_3 formation is directly controlled by the moisture level besides the temperature and pH (Elliott and Collins, 1982; Carr et al., 1990).

Chickens reared on chopped straw had lower FPD scores. In the fifth week of age, the application of Micropan led to the lowering of pH with both types of litter (chopped and unchopped).

Crust formation at the surface of the litter treated with Micropan was observed from the fourth to sixth week of the trial. It is assumed that this led to a lower FPD score in chickens reared on unchopped straw as well. The crust was dry; thus, the foot pads were cleaner, but the bottom below the litter was wetter than the bottom of the pen without Micropan. The samples used for measuring the moisture content of the litter came from the top to the bottom of the litter layer. Most likely this was the reason why there is no significant difference in moisture levels between all observed groups

This study, as well as investigations by Bilgili et al. (2009) and Shepherd and Fairchild (2010), confirms that the particle size of litter material has an important role in the development of FPD because broiler chickens kept on chopped straw had lower FPD score at all ages analysed. Hester et al. (1997) also found that the highest incidence of FPD was with the coarse-particle board treatment.

In all 3 observed weeks, ammonia concentration was not significantly influenced by the addition of Micropan or straw size. These results are not consistent with the research of Jeppsson (1999), who pointed out that chopped straw emits considerably less ammonia than long straw in a building for young cattle. Our results agree with the results of Slobodzian-Ksenicz and Kuczyński (2002), who did not find any significant effect of litter type (long straw, wood shavings, or chopped straw) on ammonia emission in turkey housing.

In this experiment ammonia concentration varied from 36.88 to 62.50 ppm at the surface of the litter in all groups. These concentrations were less than the emission rates found by Bilgili et al. (2009) (65 to 105 ppm) and greater than the emission rates reported by Do et al. (2005) (up to 39 ppm) and Witkowska et al. (2006) (up to 31.2 ppm). Iwańczuk-Czernik et al. (2007) state that the application of a microbiological preparation (Biosan-GS) and a disinfectant (Lubisan) in broilers reduced ammonia concentration in litter. Based on the ammonia levels observed in this study, this hypothesis cannot be confirmed.

5 Conclusion

A study was conducted to evaluate the impact of Micropan (microbiological product) application and the straw size on ammonia emission and footpad dermatitis in broilers. The following conclusions were drawn:

- Broilers reared on chopped straw had significantly better leg condition as expressed by a smaller incidence and severity of FPD at 3 and 5 weeks of age.
- Litter pH was decreased by Micropan application in broilers kept on chopped and unchopped straw in the fifth week of age
- There was no significant effect of Micropan application and straw size on ammonia emission in broiler housing.
- The footpad quality was significantly improved by Micropan application at 6 weeks of age.

– Further studies on commercial farming are needed to determine the effects of litter amendments and the straw size on footpad dermatitis in broilers.

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